Comparative Trial of P1496, a New Non-steroidal Oestrogen Analogue

WULF H. UTIAN

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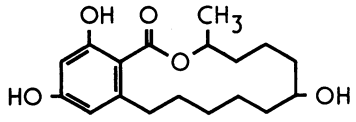
Summary

The results are reported of a preliminary trial of P1496, a new non-steroidal oestrogen analogue, compared with a conjugated equine oestrogen and a placebo. The oestrogenicity of both substances was well substantiated by vaginal epithelial maturation indices. P1496 was superior to conjugated equine oestrogen in producing a significant reduction of plasma calcium levels and a possible reduction in serum cholesterol. Conjugated oestrogen caused slightly more nausea than P1496 but there were no notable side effects from either drug. P1496 is considered to be at least as effective an oestrogenic substance as conjugated oestrogen and worthy of further therapeutic evaluation.

Introduction

This paper introduces P1496, a new drug with oestrogenic activity but unrelated to any steroidal oestrogen. Its chemical structure is shown in the figure. According to Sandoz, the manufacturers, the oestrogen activity of this non-steroid compound is weak.



In terms of its effect on the uterine growth response in mice and rats it is classified as an impeded oestrogen of the oestriol type. Oral doses of 0·9 mg/kg/day produce oestrogenic changes in ovariectomized monkeys. No progesterone-like or androgenic activity have been found nor any untoward effects that have not been observed with other oestrogenic compounds. A trial of P1496 in women was therefore thought to be justified, and the results are reported here of a study of some of its clinical and metabolic effects compared with those of conjugated equine oestrogens and a placebo.

Subjects and Methods

The volunteer subjects, 45 in number, were healthy women, aged from 45 to 56 years and of similar socioeconomic status, who had undergone total abdominal hysterectomy with bilateral oophorectomy within three years of being admitted to the study. The subjects were separated into three groups of 15 and each group was given three consecutive treatment cycles (three weeks on and one week off therapy), one group being given P1496 75 mg daily, another conjugated equine oestrogen 1·25 mg daily, and the third placebo. All patients were assessed clinically at the end of each three-week period of treatment and finally three weeks after stopping treatment. Specimens for laboratory analysis were taken at the start of the study and again at the end of the third cycle of treatment.

Details of symptomatology (hot flushes, dyspareunia, blood-stained vaginal discharge, pruritus vulvae), changes of clinical signs (body weight, blood pressure), and evaluation of side effects (nausea, vomiting, breast discomfort, oedema, leg cramps, and thromboembolism) were determined according to accepted clinical criteria. Plasma cholesterol, calcium, inorganic phosphorus, alkaline phosphatase, serum protein bound iodine (P.B.I.), haemoglobin, packed cell volume, and vaginal smear for hormonal cytology were determined by standard methods.

The severity of hot flushes was determined by the average number of flushes a day. Fewer than three was classified as mild, four to six as moderate, and more than seven as severe. These groups were given scores of 1, 2, and 3 respectively and multipled by a factor of 6 to give an assessment allowing of statistical analysis (Kupperman *et al.*, 1953).

Vaginal smears for hormonal cytology were taken with an Ayre's spatula from the upper third of the right lateral vaginal wall and immediately fixed and stained by the method of Papanicolaou. A total of 500 cells were counted and the maturation index obtained.

Plasma concentrations were measured by the following methods. Cholesterol by the method described by Pearson *et al.* (1953), as modified by Duncan (1959) (normal 150-250 mg/100 ml). Calcium by high atomic absorption specrophotometry (normal 8·5 to 10·6 mg/100 ml). Inorganic phosphorus by the standard clinical method of Fiske and Subarrow (1929) (normal 2·8 to 4·5 mg/100 ml). Alkaline phosphatase by autoanalyzer using phenolphthalein monophosphate as substrate with results corrected to Bodansky-Reinhart units (normal less than 12 B.R.U.).

Serum protein bound iodine estimations were made by automatic digestion on the Technicon AutoAnalyzer (Riley and Gochman, 1964).

Results

Hot Flushes.—The average score for hot flushes for each treatment group is shown in table I. Both P1496 and conjugated oestrogen significantly reduced the incidence and severity of hot flushes ($P < 0·01$). The placebo produced no response. There was no significant statistical difference in effect between the two oestrogens used.

TABLE I—*Kupperman Average Score for Hot Flushes for the Three Treatment Groups*

Treatment Group	Average Kupperman Score				
	Before Treatment	Third Week	Seventh Week	Eleventh Week	After Treatment
P1496	14·1	5·1	4·3	3·4	15·4
Conjugated Oestrogen	9·6	3·0	0·6	0·6	9·0
Placebo	9·2	8·8	10·2	9·7	11·1

Department of Gynaecology, Groote Schuur Hospital and University of Cape Town, South Africa
WULF H. UTIAN, PH.D., M.R.C.O.G., Consultant and Lecturer

TABLE II—*Percentage of Parabasal, Intermediate, and Superficial Cells in Vaginal Smear of Patients in the Three Groups Before, During, and After Treatment*

Treatment Group and Type of Cell	No. of Patients	Percentage No. of Cells				
		Before Treatment	During First Cycle	During Second Cycle	During Third Cycle	After Treatment
P1496:						
Parabasal	14	7·6	5·2	0·0	1·0	1·0
Intermediate	14	88·3	92·2	83·7	80·6	94·8
Superficial	14	4·1	2·6	16·3	18·4	3·2
Conjugated Oestrogen:						
Parabasal	15	14·9	1·1	0·0	1·1	4·3
Intermediate	15	82·9	96·7	96·3	92·9	93·9
Superficial	15	2·2	2·2	3·7	6·0	1·8
Placebo:						
Parabasal	12	13·6	12·8	14·7	7·1	5·9
Intermediate	12	80·7	86·2	83·5	92·6	94·0
Superficial	12	5·7	1·0	1·8	0·3	0·1

Dyspareunia, Blood-stained Discharge, and Pruritus Vulvae.—Two out of three patients in the P1496 group who complained of dyspareunia before treatment obtained significant relief on the drug, and a blood-stained discharge in one of these patients had disappeared by the third week of therapy. A similar response was noted in the conjugated oestrogen group, dyspareunia in one patient and a blood-stained discharge in three being cured by the third week of therapy. There was no response in two patients in the placebo group who presented with dyspareunia and blood-stained vaginal discharge. No patient in any group had pruritus vulvae.

Body Weight and Blood Pressure.—There were no significant changes in blood pressure or body weight in the patients in any of the groups, the results at the 11th week being compared statistically with the pretreatment value.

Side Effects.—The only significant side effects were nausea and breast discomfort. Four cases of each occurred in the conjugated oestrogen group, while one patient in the P1496 and two in the placebo group complained of nausea. No patients showed signs of deep or superficial vein thrombosis.

Vaginal Cytology.—The effect on the vaginal epithelial cell types in the three treatment groups is summarized in table II. Both oestrogens promoted the maturation of vaginal epithelial cells from the parabasal to the intermediate and superficial cell types. These results are statistically significant when the relative parabasal cell counts are compared against the placebo group ($P < 0.01$) but not when the superficial and intermediate cell counts are compared. Though the effect of P1496 on the superficial cell count was numerically superior, the statistical comparison between P1496 and the conjugated oestrogen shows no significant difference.

Packed Cell Volume.—There was no significant change in packed cell volume or haemoglobin in any of the groups.

Serum Protein Bound Iodine.—Unexpectedly, no significant changes in P.B.I. were produced by either form of oestrogen.

Plasma Cholesterol, Calcium, Phosphorus, and Alkaline Phosphatase.—P1496 produced a slight decrease in the plasma cholesterol value at the end of three cycles of therapy, a reduction of 24 mg/100 ml in the mean (table III) being of only possible significance ($P < 0.05$). There was no significant change in mean plasma cholesterol values with conjugated oestrogen. There was a significant reduction in the mean value of plasma calcium from 10·38 to 9·65 mg/100 ml (a difference of 0·7) on P1496 therapy ($P < 0.01$) (table III). There were no changes of significance on conjugated oestrogen or placebo therapy. Except for a marginal rise in levels of plasma inorganic phosphorus on P1496 therapy ($P < 0.05$) there were no other significant changes. All alkaline phosphatase values were within normal limits, showing that none of the patients had any manifest form of bone disease. No significant changes were produced on oestrogen or placebo therapy.

TABLE III—*Differences in Mean Values (mg/100 ml) and Percentage Changes from Initial Values of Plasma Cholesterol, Calcium, and Phosphorus in the Three Groups at End of Third Treatment Cycle*

Substance and Treatment Group	Difference in Mean (mg/100 ml)	Change (%)	Significance
Calcium:			
P1496	−0·7	7	$P < 0.01$
Conjugated Oestrogen	−0·3	3	N.S.
Placebo	+0·3	3	N.S.
Inorganic Phosphorus:			
P1496	+0·4	10	$P < 0.05$
Conjugated Oestrogen	−0·2	6	N.S.
Placebo	+0·2	5	N.S.
Cholesterol:			
P1496	−24	9	$P < 0.05$
Conjugated Oestrogen	−4	1	N.S.
Placebo	+5	2	N.S.

N.S. = Not significant.

Discussion

The only symptoms directly associated with endogenous oestrogen withdrawal and specifically relieved by replacement therapy are hot flushes and those related to atrophic vaginitis. Other symptoms such as depression, irritability, and insomnia respond significantly to placebo therapy and are therefore most likely of psychological origin (Utian, 1972a). On this basis both P1496 and conjugated oestrogen showed significant oestrogenic activity. Both were equally effective in alleviating hot flushes, and both significantly reduced the number of parabasal cells present in the vaginal smear.

The absence of change in P.B.I. was unexpected in view of the known change in P.B.I. found in pregnancy, thought to be induced by oestrogen (Dowling *et al.* 1956), and in normal subjects when given stilboestrol, oestradiol benzoate, or mestranol (Dowling *et al.* 1960; Gow and MacGillivray, 1971). The study confirmed the observation that serum cholesterol values are slightly reduced by oestrogen therapy, though not all oestrogens have this effect (Utian, 1972b). Plasma calcium changes with oestrogen therapy are important because of the possible relationship to the development of osteoporosis (Nordin, 1971; Utian, 1971). P1496 produced a significant reduction in plasma calcium, and this oestrogenic effect has been shown by Frost (1961) and Lafferty *et al.* (1964) to be due primarily to reduced bone resorption. Conjugated oestrogens have been shown to be very effective in reducing plasma calcium levels (Utian, 1972c), and their failure to do so in the present investigation cannot be explained. There were, moreover, minimal changes in the plasma inorganic phosphorus levels with both oestrogens used. The alkaline phosphatase values were included only for completeness.

The absence of any venous thromboembolic disease in this series contrasts with the exceptionally high incidence (16%) in that of Gow and MacGillivray (1971). They gave mestranol, an unconjugated synthetic oestrogen, and that may account for the difference. Nevertheless, a possible thrombogenic effect of P1496 should be investigated further. Though unconjugated

steroidal oestrogens may be more thrombogenic than natural conjugated oestrogens (M. Notelowitz, personal communication, 1972) the safety of non-steroidal over steroidal oestrogens has yet to be proved. Moreover, the relation between any oestrogen and thromboembolic disease has yet to be fully elucidated (Drill and Calhoun, 1972).

This preliminary study shows that P1496 is a satisfactory oestrogenic preparation without any notable side effects. With increasing knowledge of the endocrinological changes at the menopause and their relation to pathological changes there is need for a strict evaluation of all available oestrogenic substances, including P1496.

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Modification of Plasma Corticosteroid Concentrations during and after Surgery by Epidural Blockade

N. H. GORDON, D. B. SCOTT, I. W. PERCY ROBB

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Summary

The adrenocortical response to major surgery was assessed by the measurement of plasma corticosteroid concentrations in patients receiving either a general anaesthetic alone or in combination with an epidural block. The expected increase in plasma corticosteroid concentration seen in the general anaesthesia group was significantly inhibited by epidural block. The inhibition was related to the duration of the epidural block. The lack of the normal adrenocortical response to surgery was not associated with cardiovascular collapse and indeed did not seem detrimental in any way. These findings question the need for corticosteroid "cover" for the stress of surgery in patients who have been taking corticosteroid drugs.

Introduction

In response to major surgical procedures there is a rapid and sustained increase in the concentrations of plasma corticosteroid (Carter and James, 1970), corticotrophin (ACTH), and growth hormone (Ichikawa *et al.*, 1971). A variety of stressful situations are known to increase the plasma corticosteroid concentration, among them preoperative apprehension. The main stimulus is, however, undoubtedly the surgical procedure itself.

University of Edinburgh, Edinburgh EH8 9YL
N. H. GORDON, M.B., F.F.A. R.C.S., Lecturer in Anaesthesia
I. W. PERCY ROBB, M.B., M.R.C.PATH., Senior Lecturer in Clinical Chemistry

Royal Infirmary of Edinburgh, Edinburgh EH3 9YW
D. B. SCOTT, M.D., F.F.A. R.C.S., Consultant Anaesthetist

Painful stimuli cause a rapid rise in plasma corticosteroid concentration which may be partly blocked by hypnotically suggested anaesthesia (Black and Friedman, 1968). The response is, however, not affected by deep ether anaesthesia (Oyama *et al.*, 1968) or by spinal block used alone (Johnston, 1964).

We have studied the effects of epidural blockade in combination with general anaesthesia on the adrenocortical response to major surgery.

Method

Eighteen women undergoing abdominal hysterectomy were studied, whose only abnormalities were gynaecological. They were divided into three consecutive groups of six—group A receiving general anaesthetic alone, group B receiving general anaesthetic plus a single dose epidural block, and group C receiving general anaesthetic and a prolonged epidural block. All surgery was performed before 11 a.m. In group A, receiving general anaesthetic only, tubocurarine 30 mg was given before tracheal intubation and the respiration controlled. The two other groups were allowed to breathe spontaneously without intubation. Lumbar epidural block was performed in the lateral position (20 ml 2% lignocaine). Group C had an epidural catheter inserted, and 90 minutes after the initial injection they were given 10 ml of 0·5% bupivacaine (without adrenaline). This produced sensory loss for up to four hours—that is, well into the postoperative period.

Venous blood (control sample) was withdrawn just before premedication (intramuscular diamorphine 5 mg and atropine 0·6 mg) which was given one hour before surgery. A further blood specimen was taken just before induction of general anaesthesia (thiopentone 500 mg followed by nitrous oxide, oxygen, and 1% halothane).

Further blood specimens were obtained immediately before the surgical incision and at 15-minute intervals during the operation. The length of operation was from 60 to 100 minutes